A study of methemoglobin levels in infants from birth through six months showed that even healthy babies not exposed to excessive nitrate levels in diets have higher levels when young. Babies with diarrhea or respiratory illness had the highest levels in this population. Ingestion of water or formula high in nitrates appears to increase the frequency of elevated methemoglobin. More than 60% of formulae showed bacterial contamination. Long-term consequences should be investigated.

Methemoglobin Levels in Infants in an Area With High Nitrate Water Supply

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Introduction

Elevated nitrate levels in community water supplies are of concern because infant methemoglobinemia has been associated with the intake of water with high nitrate concentrations. This was first recognized clinically in 1945.[1] As methemoglobinemia is not a reportable disease in the United States, it is difficult to accurately determine the incidence, but there have been no reported fatalities in the United States since 1960. Since 1945, it is estimated that between 1,500 and 2,000 cases have occurred throughout the world. Because of these findings, the U. S. Public Health Service recommended a drinking water standard of 45 mg/l of nitrate (or equivalent to about 10 mg/l of nitrate nitrogen).[2]

The factors responsible for elevated nitrate contents in well water sources include geography, geology, groundwater hydrology, and the addition of nitrates naturally and from surface contamination by nitrogenous fertilizers or by organic waste of human or animal origin.

No clinical cases of methemoglobinemia have been reported in California. Few or no cases have been reported from any other part of the United States where the water used was from a community water supply. Nearly all of the cases reported in the United States were from rural households with water supplies from wells of questionable sanitary construction. This suggests that heavy bacterial contamination might also have been present. Surveillance of domestic groundwater supplies in California indicates that several communities have community water supplies with nitrate levels exceeding the Public Health Service Drinking Water Standards.

In contrast to the overt clinical cases, little is known or has been reported concerning the subclinical effects from the use of high nitrate waters for infant feeding. We have no information concerning whether health effects do occur, or what they might be when an infant has a high nitrate intake from birth. Nitrate in food or water becomes a hazard to health when the nitrate is converted to nitrite, and this ion when absorbed converts hemoglobin to methemoglobin. The conversion may be carried on by bacterial contaminants, or by bacteria in the digestive tract. Some methemoglobin is present in the normal healthy infant but we do not know whether there is a problem when subclinical elevations of methemoglobin occur in infants under six months of age.

On the basis of experimental studies[3] it is thought that the methemoglobin level fluctuates in response to absorption of nitrite ion and the activity of the enzyme methemoglobin reductase, which reconverts methemoglobin to hemoglobin. It has been suggested that this enzyme is less active in younger babies (less than three months of age) or in those with deficiency of glucose-6-phosphate dehydrogenase.

Bodansky[4] has reported on some of the possible health implications of elevated nitrate ingestion and Knotek and Schmidt[5] in Czechoslovakia have presented data on the effect of differences in the type of dried milk formula (regular and buttermilk), as well as the apparent role of bacteria interacting with the acidity of the formulas. Studies in cattle fed high nitrate and high nitrite diets, have shown inhibition of growth and shortening of the median life span,[6] significant reduction in Vitamin A,[7] increased abortion rate, reproductive difficulties, reduced milk production, and a poor utilization of Vitamin A.[8]

Recent studies by Gruener and Shuval[3] have shown the nitrite ion, when fed to pregnant rats as sodium ni*trite* ($NaNO_2$), could be transmitted across the placenta, resulting in impaired growth. These investigators have also found abnormalities of the EEG in animals given high doses of sodium nitrite. The evidence of such effects in animals, emphasizes the need to study the long-term health implications of subclinical elevations of methemoglobin for infants and for pregnancy.

Methods

The primary area of study included two communities of 15,000 and 5,000 population, five miles apart, in the south central area of California known to use groundwater with varying levels of nitrate. The area is at an elevation of a little over 350 feet, the rainfall averages 6.44 inches per year and the temperature ranges from an average low of 38°F during the winter months to an average of 90°F in the summer months. The economy of the area is dependent on agriculture, particularly the production and shipping of cotton, grapes, field crops, and citrus fruits. In 1967, nitrate concentrations in the groundwaters in this vicinity were studied,[9] considering in detail the sources, seasonal variations and water table variations.

Infants from one to six months were examined, and the nitrate-nitrite intake from their formula and water was determined along with an analysis for bacterial contamination of water and formula. In the primary study area, the well waters were tested weekly.

All infants born in the area were invited to clinics for an interview and methemoglobin determination. Clinics were scheduled twice a month. During months when both parents worked, evening as well as day clinics were held. All families were sent appointments, contacted by a health worker before the clinic and afterwards if an appointment was missed. The infant was seen at one month of age and at two, three, four and six months of age when possible.

The interview included a 24-hour dietary intake history of food and formula, a water intake history and an illness history. Methemoglobin and hemoglobin levels were determined from capillary blood samples, and the home of each infant was visited the day of the clinic visit to collect samples of the water supply used by the family and the water and formula used by the infant. Water samples for bacterial counts were taken from home faucets when they were first opened, as well as after flushing for a few minutes. Assessment of findings is based on the opinion that the nitrate content of water at the home is more of a controlling factor than nitrate content of the well sources.

All samples were analyzed at the California State Department of Public Health for nitrate and bacterial concentration. Examination of water and formula used by infants included a determination of nitrite concentration. Because of the instability of methemoglobin in whole blood, its analysis should be started within half an hour. Laboratory equipment and reagents were brought to the clinic by a chemist who measured methemoglobin and hemoglobin concentrations immediately after blood samples had been taken.

Participating infants were given general physical examinations including growth and development measurements at the end of the study.

The study was discussed with physicians practicing in the community prior to the clinic visits. All findings were discussed with the parent and were, in addition, sent to the infant's physician.

Infants receiving prepared formula or bottled water, or those being breast-fed formed a suitable control population within the service area of the community water supply.

Laboratory Methods

Hemoglobin and methemoglobin concentrations were determined on 0.1 ml samples of capillary blood taken by heel prick during the interview. The method used was based on that published by Hainline[10] with modifications suggested by Winton and Tardiff[11] and Hegesh, et al.[12] It was necessary to extend the duration of centrifugation following hemolysis in Tritonborate solution in order to insure a clear supernatant solution.

Water and formula samples were iced or preserved and sent to the headquarters laboratory for determination of nitrate concentration by the brucine method[13] and for estimation of coliform, fecal coliform, and total bacterial densities. Nitrate-nitrite concentration in milk or formula was determined by a method published by Manning, et al.[14] Nitrite in water was determined following the method of Strickland and Parsons.[15] Coliform and fecal coliform densities (as Most Probable Number, MPN) were estimated by use of the multiple tube fermentation method.[13] A standard plate count (SPC) was also performed on each sample to provide an assessment of the non-coliform contamination levels.

Results

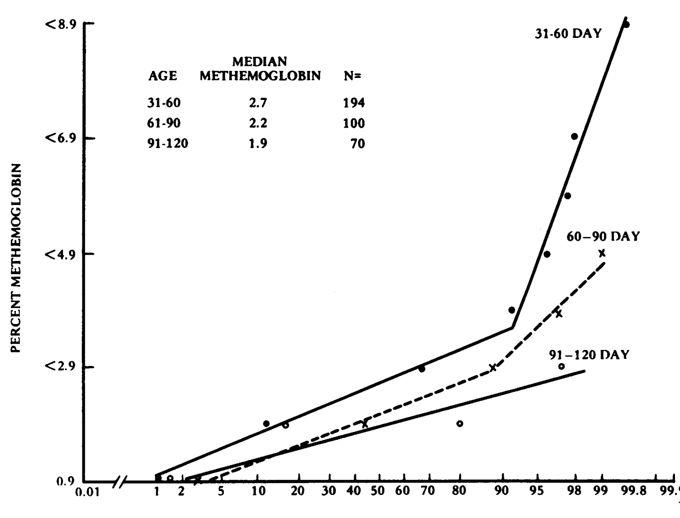
Slightly more than half the infants born in the area were examined at least once. Those who participated and those who did not were similar in ethnic origin and in area of residence. While there may be a selection bias, we were unable to identify any pattern which could be influenced by it.

Half the infants in the nitrate study were on concentrated formula, usually diluted with an equal volume of water. Twenty-four per cent used prepared formula, and ten per cent were on cows' milk to which no water was added. Nine per cent were on evaporated milk formulas with varying concentrations although mostly 1:1 dilutions. Less than three per cent were on dry milk formula and less than three per cent were breast-fed. Commercial formulas generally contain 50 mg/l of Vitamin C, the infant thus receiving 1.5 mg per ounce of formula per day.

Over a period of one year, 487 examinations were completed for 256 infants. One-hundred seventeen of these infants had multiple visits at different ages. Male and female infants were equally distributed in the study group and there did not appear to be any significant difference in methemoglobin levels by sex.

There were age variations in methemoglobin levels, independent of water supply. Figure 1 and Table 1 show that young babies, up to 60 days, who are in good health, are likely to have higher methemoglobin levels than do older babies. This effect appears to be independent of the level of nitrate in water, since it is also found among babies who are breast-fed or whose formulas are made with bottled water.

Figure 1—Cumulative Per Cent of Infants by Age Groups for Per Cent Methemoglobin March 1970-March 1971



The cumulative frequency distribution by age, Figure 1, shows a bimodal distribution, of which the lower portion is below 4% methemoglobin. Accordingly, we have divided the results into those with less than 4% methemoglobin and those with greater. Those with more than 4% methemoglobin we consider to have elevated levels. This occurred in twenty-one infants, one of whom had an elevated level on two successive examinations. There are thus twenty-two positive tests or 4.5% positive.

Babies who have minor illness appear to have higher methemoglobin levels than healthy babies of the same age. One-third of the infants with elevated values (above 4%) had respiratory illness, but the four highest values observed were from infants with diarrhea. Tables 4, 7 and 8.

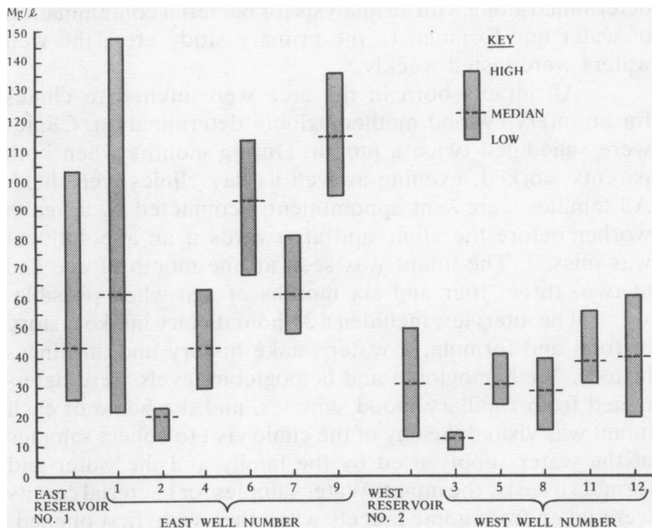
Since it is anticipated that standards for the community water supplies might be based on the total nitrate-nitrite-nitrogen content, the intake of infants was calculated similarly on the basis of total nitrate-nitrite-nitrogen intake in milligrams per liter. This was calculated from the history of ingestion and the measured levels in water and formula. This approach differs from that used by Winton, Tardiff, and McCabe.[16] Table 2 shows the relationship of methemoglobin levels to nitrogen intake by age. Table 3 shows the level of methemoglobin for babies who gave no history of illness. While elevated methemoglobin values (>4%) are about three times as frequent for not ill babies with high nitrogen intake as compared to those with low nitrogen intake, the difference is not statistically significant with this sample size. Among ill infants, elevated methemoglobin levels are five times as frequent in the 5.0 to 9.9 mg per day nitrogen intake group as those with an intake below 5 mg.

Bacteria in significant numbers were found in the water and formulae available to infants based on coliform, fecal coliform and standard plate counts, Table 5. Immediate and flushed samples from the household supply, bottled water, boiled tap water, and the formula often revealed considerable contamination. The least contamination was found in the immediate and flushed samples of the public community water supply.

The various types of commercial formula used by the families were analyzed and all had less than 4.0 mg/l of nitrate and less then 0.05 mg/l of nitrite. Dextrose was used in evaporated milk formula, and this had a level of 0.52 mg/l of nitrate and less than 0.05 mg/l nitrite. Boiling of water for formula for up to 10 minutes produced little change in the concentration, but between 10 and 15 minutes nitrate-nitrogen concentrations began to increase. In a laboratory experiment ni*trite* was produced in greater amounts when the nitrate-containing water was boiled in aluminum, rather than in glass. These observations require further study.

The wells serving the Western side of the larger town have a relatively low nitrate level, whereas those serving the Eastern side of the town fluctuate as shown in Figure 2. On the side with lower nitrate levels, no single well exceeded the 45 mg/l limit, but on the other side, demand for water occasionally required using a well with higher concentrations. Nitrate concentrations in the water from any single well are known to be subject to variation by time and season. Tables 6a, b, and c show the relationship of methemoglobin levels to nitrate exposure by area by age. Tables 7 and 8 list the circumstances in which elevated methemoglobin levels (4.3%) were observed in 21 infants. Table 9 shows the reversibility of elevations when babies were changed from water with a high nitrate-concentration to bottled water.

Figure 2—Well Nitrate Concentrations, High-Low-Median, March 1970-March 1971



The physical examinations on 98 infants did not reveal any findings different than the expected normal variations.

Discussion

Prior studies have shown that only very young babies, usually less than 60 days, became ill with methemoglobinemia and that bacterial contamination was probably necessary to produce this clinical illness. Since we have observed no cases of clinical illness, we can only provide peripheral information concerning these problems. However, many variables play an important role in the determination of methemoglobin levels. The role of age is seen in the clustering of elevated values in the 31-60 day group. No infant over 90 days had a methemoglobin over 3.0 per cent. The majority of illness was due to respiratory infections, however, the highest methemoglobin elevations were in infants with diarrhea. For example one infant had 30 days of loose stools. This infant had a high nitrate exposure and the highest methemoglobin level, 10.7%. Among the other infants there was no consistent correlation between nitrogen-intake and methemoglobin elevation. The only infant hospitalized with diarrhea had a methemoglobin level of 8.4 per cent but had been using bottled water continually.

The presence of bacteria in water and formula may present as much of a problem as the presence of nitrate. Table 5 clearly indicates the need for better education of parents in the handling of infant feeding.

Taken singly the "risk factors" for elevated methemoglobin associated with 1) high nitrate ingestion (more than 5 mg. in 24 hours). 2) illness, 3) location and 4) contamination of formula by fecal coliform organisms are shown in the following contingency tables.

The χ^2 of 9.7 for nitrate ingestion in the table is significant at the 1% level. Because of the small sample size, this was confirmed by using Fisher's exact test. However, the

	Nitrate Ingestion 0-5 mg	> 5mg	Total
Mhgb<4%	410	53	463
Mhgb ≥4%	14	8	22
χ^2	9.7		

	Illness Status Not Ill	Ill	Total
Mhgb <4%	359	104	463
Mhgb ≥4%	14	8	22
χ^2	n.s.		

	Location East	Other	West	Total
Mhgb<4%	159	131	174	464
Mhgb≥4%	5	8	9	21
χ^2	n.s.			

	Fecal Coliform Count in Formula <30	>30	Total
Mhgb<4%	165	253	418
Mhgb≥4%	5	17	22
χ^2	n.s.		

five highest methemoglobin tests occurred in babies with illness, only one of whom had an elevated nitrate ingestion.

These findings represent a set of observations of a rapidly fluctuating set of phenomena. They may not be representative of the maximal potential of these variable processes.

Conclusion

Our data indicate that even healthy babies not exposed to excessive nitrate levels in their diets, have higher methemoglobin levels when they are young, that is, under 60 days, than they do when they are older. We also find the highest levels of methemoglobin (over 6% MHb) in babies who have respiratory illness or diarrhea. Since the babies which we examined were not acutely ill, it is conceivable that acutely ill babies have a much more serious problem from methemoglobinemia.

Compared to the effects of age and state of health in elevation of the methemoglobin level (over 4% MHb) the effect of ingesting nitrate-nitrite-nitrogen in excess of 5 mg. per day from community water supplies is detectable but not impressive. Bacterial contamination of formula may contribute to such elevations.

Table 1—Methemoglobin Levels By Age March 4, 1970 thru March 25, 1971

Methemoglobin per cent	Total		Age in days 0 - 30		31-60		61-90		91-120		>120	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0.0-0.9	6	1.2	-	—	2	1.0	3	3.0	1	1.4	-	-
1.0-1.9	178	36.6	7	15.2	21	10.8	39	39.0	55	78.6	56	72.7
2.0-2.9	214	43.9	28	60.9	107	55.2	46	46.0	12	17.1	21	27.3
3.0-3.9	68	14.0	9	19.6	48	24.8	9	9.0	2	2.9	-	—
4.0-4.9	12	2.5	2	4.3	8	4.2	2	2.0	-	—	-	-
5.0-5.9	4	.8	-	—	3	1.5	1	1.0	-	—	-	-
6.0-6.9	1	.2	-	—	1	.5	-	—	-	—	-	-
7.0-7.9	-	—	-	—	-	—			-	—	-	-
8.0-8.9	3	.6	-	—	3	1.5	-	—	-	—	-	-
9.0-9.9	-	—	-	—	-	—	-	—	-	—	-	-
10.0-10.9	1	.2	-	—	1	.5	-	—				
Total	487	100.0	46	100.0	194	100.0	100	100.0	70	100.0	77	100.0

Table 2—Nitrate-Nitrite Nitrogen Ingestion and Methemoglobin Levels by Age for Total Infants

		Twenty-four hour nitrogen intake <5 mg		5.00-9.99 mg		10.0-20.0 mg	
	No. Infants	Mhgb level		Mhgb level		Mhgb level	
Age	examined	<4%	≥4%	<4%	≥4%	<4%	≥4%
< 30 days	45	38	2	5	0	0	0
31-60 days	194	156	11	19	6	2	0
61-90 days	102	87	1	11	2	1	0
91-120 days	68	64	0	2	0	2	0
>120 days	76	65	0	10	0	1	0
Subtotals	485	410	14	47	8	6	0
Totals		424		55		6	
Per cent with 4% Mhgb or more*		3.3%		14.5%		0.0%	

*4.52% of the Total Infant Population have Mhgb above 4%.
5 mg/l Nitrogen=22.15 mg/l NO_3
10 mg/l Nitrogen=44.3 mg/l NO_3

Table 3—Nitrate-Nitrite Nitrogen Ingestion and Methemoglobin Levels by Age for Infants Without Illness

		Twenty-four hour nitrogen intake					
		<5 mg		5.00-9.99 mg		10.0-20.0 mg	
	No. Infants	Mhgb level		Mhgb level		Mhgb level	
Age	examined	<4%	≥4%	<4%	≥4%	<4%	≥4%
<30 days	37	30	2	5	0	0	0
31-60 days	156	128	6	17	3	2	0
61-90 days	81	68	1	9	2	1	0
91-120 days	51	48	0	1	0	2	0
> 120 days	48	40	0	7	0	1	0
Subtotals	373	314	9	39	5	6	0
Totals		323		44		6	
Per cent with 4% Mhgb or more		2.78%		11.3%		0.0%	

Table 4—Nitrate-Nitrite Nitrogen Ingestion and Methemoglobin Levels by Age for Infants with Diarrhea and Respiratory Illness

		Twenty-four hour nitrogen intake					
		<5 mg		5.00-9.99 mg		10.0-20.0 mg	
	No. Infants	Mhgb level		Mhgb level		Mhgb level	
Age	examined	<4%	≥4%	<4%	≥4%	<4%	≥4%
<30 days	8	8	0	0	0	0	0
31-60 days	38	8	5	2	3	0	0
61-90 days	21	19	0	2	0	0	0
91-120 days	17	16	0	1	0	0	0
>120 days	28	25	0	3	0	0	0
Subtotals	112	96	5	8	3	0	0
Totals	112	101		11			
Percent with 4% Mhgb or more		4.9%		27.2%			

Table 5—Fecal Coliform Water and Formula Samples March 1970-March 1971

		Fecal coliform (E.C.)			
		Acceptable*		Not Acceptable†	
Sample	Total No.	No.	%	No.	%
Immediate	481	439	91.3	42	8.7
Flushed	476	458	96.2	18	3.8
Tap boiled	253	204	80.6	49	19.4
Bottled	153	98	64.1	55	35.9
Formula	440	170	38.6	270	61.4

Fecal coliform organisms
*<3 Water
<30 Formula
†3 & > Water
30 & > Formula

Table 6a—Nitrate-Nitrite Nitrogen Ingestion and Methemoglobin Levels by Location for Total Infants

		East Delano			
		Twenty-four hour nitrogen intake			
		<4.99 mg		5.00+ mg	
	No. Infants	Mhgb level		Mhgb level	
Age	examined	<4%	≥4%	<4%	≥4%
<30 days	14	12	0	2	0
31-60 days	63	54	1	6	2
61-90 days	40	34	1	4	1
>90 days	47	46	0	1	0
Subtotals	164	146	2	13	3
Totals		148		16	
Per cent with 4% Mhgb or more*		1.4%		18.8%	

*4.52% of the Total infant population have Mhgb above 4%.
5 mg/l Nitrogen=22.15 mg/l NO_3
10 mg/l Nitrogen=44.3 mg/l NO_3

Table 6b—Nitrate-Nitrite Nitrogen Ingestion and Methemoglobin Levels by Location for Total Infants

		West Delano			
		Twenty-four hour nitrogen intake			
		<4.99 mg		5.00+ mg	
	No. Infants	Mhgb level		Mhgb level	
Age	examined	<4%	≥4%	<4%	≥4%
<30 days	15	14	1	0	0
31-60 days	76	58	8	10	0
61-90 days	36	31	0	5	0
> 90 days	56	50	0	6	0
Subtotals	183	153	9	21	0
Totals		162		21	
Per cent with 4% Mhgb or more*		5.55%		0.00%	

*4.52% of the Total Infant Population have Mhgb above 4%.
5 mg/l Nitrogen=22.15 mg/l NO_3
10 mg/l Nitrogen=44.3 mg/l NO_3

Table 6c—Nitrate-Nitrite Nitrogen Ingestion and Methemoglobin Levels by Location for Total Infants

		Other locations			
		Twenty-four hour nitrogen intake			
		<4.99 mg		5.00+ mg	
	No. Infants	Mhgb level		Mhgb level	
Age	examined	<4%	≥4%	<4%	≥4%
<30 days	16	12	1	3	0
31-60 days	55	44	2	5	4
61-90 days	26	22	0	3	1
>90 days	42	34	0	8	0
Subtotals	139	112	3	19	5
Totals		115		24	
Per cent with 4% Mhgb or more*		2.6%		20.8%	

*4.52% of the Total Infant Population have Mhgb above 4%.
5 mg/l Nitrogen=22.15 mg/l NO_3
10 mg/l Nitrogen=44.3 mg/l NO_3

Table 7—Illness By Age With 4.0% Methemoglobin March 1970-March 1971

		Age in days						Type of Illness				
Mhgb	No.	0-30	31-40	41-50	51-60	61-70	71-80	Resp	Diar	None	Med	Unk
4.0-4.9	12	2	2	4	2	1	1	4	1	4	2	1
5.0-5.9	4			3			1	2		2		
6.0-6.9	1		1					1				
7.0-7.9				1								
8.0-8.9	3		2						3*			
9.0-9.9												
10.0-10.9	1		1						1†			
Total	21	2	6	8	2	1	2	7	5	6	2	1

*1 Diarrhea with respiratory illness
1 Diarrhea with medication
†Loose stools for 30 days

Table 8—Comparison of Birth Weight, Illness, Formula, Water and Nitrogen Intake for Five Highest Methemoglobins

%	Birth		Formula				Water intake	NO_3-NO_2
Mhgb	weight	Illness	Type	Oz.	Contamination	Type	Oz.	Nitrogen/24 hr
6.1	7-5	R*	Evap	11¼	X‡	Term. Steril	0	2.89
8.9	7-6	D*	Prep	12	X	Bottled	0	0.17
8.9	7-6	D	Conc 1:1	14	X	Bottled	0	0.04
8.4	7-3½	D	Dry	18	X	Tap	3½	1.16
10.7	9-6½	D†	Evap Dext	19.3	X	Tap Boiled	8	6.31

*R=Respiratory
D=Diarrhea

†Diarrhea, loose stools for 30 days
‡X=Contaminated

Table 9—Comparison of Methemoglobin Levels After 24 Hours Use of Bottled Water* and After One Month on Former Routine

Clinic Visit-1							Clinic Visit-2			
Age Days	Mhgb % CV-1	Mhgb % 24 hr†	Hgb CV-1	Hgb 24 hr†	Illness	NO_3-NO_2 Nitrogen	Age Days	Mhgb	Illness	NO_3-NO_2 Nitrogen
35*	10.7	4.03	11.6	11.1	Loose Stools x 30 days	6.306	57	3.36	—	0.049
40	2.13	2.35	10.5	9.85	0	1.296	—	—	—	—
42	2.26	2.90	9.3	9.9	0	6.965	—	—	—	—
46	5.23	3.19	11.4	11.2	0	7.762	—	—	—	—
46	8.2	2.14	10.43	9.1	Diarrhea x 2 days	1.160	74	3.57	Resp x 7 days	1.098
51	2.63	1.57	9.5	10.2	0	6.170	79	1.93	—	8.243
70	2.68	1.56	10.0	10.3	0	4.883	105	1.74	Resp x 2 days	0.597

*Except for 35 day old infant—Mhgb or type of water used not known when mothers asked to participate.
†After 24 hours on bottled water.

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